

Evaluating The Histopathological Studies In Native Fishes Thriving In The Coal Mining Areas Of Jharkhand - A Case Study

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Abstract:

Coal mining generates huge quantity of toxic effluent which consistently pollutes the neighboring wetlands where the local inhabitants regularly cultivate edible fishes. The current study aims to Estimated daily intake of heavy metals by fish and its correlation on human health. The 5 Samples were collected from Konar Dam (S1), Maithon Dam (S2), Panchet Dam (S3), Tenughut Dam (S4) and Tilaya Dam (S5) near Damodar River basin, Jharkhand (india). Mainly *P. indicus*, *M. gulio*, *P. conchoni*, *L. calbasu*, *L. rohita*, and *L. bata* Fish species were collected. Fish species *P. indicus* (F1), *M. gulio* (F2), *P. conchoni* (F3), *L. calbasu* (F4), *L. rohita* (F5), and *L. bata* (F6) are Pb, Co, Cd, and As found at low dietary intake. The maximum EDI observed only accounts for 0.11 µg/kg for F1 at dam 3, 0.35 µg/kg for F3 at dam 3, 0.22 µg/kg for F3 and F6 at dam 2, and 0.17 µg/kg for F1 at dam 3, respectively. The accumulation of heavy metals in tissues appears to cause remarkable histopathological alterations in skin, gills, liver and kidney that might be leading to deleterious effect on fish physiology and consequently impact the consumers of such fishes. The risks to our health and the environment posed by coal mining are ruining our lives. Surface water and sedimentation are found to be correlated, and the histopathology's scanning electron microscopy is also acquired.

Keywords: Coal Mining; Correlation; histopathology; Sedimentation; Fish; Human health

INTRODUCTION

The whole world is facing the problem of water quality crisis may be due to overpopulation, industrialization, and agricultural development. According to WHO [1], over 2 billion population are living in water stressed countries. India houses the world's 18% of the total population. The country is enriched with several rivers and lakes and the population has access to 4% of the world's water resources [2]. According to World Resources Report, 2017, about 70% of the water supply in India is severely polluted with sewage effluents [3]. In India, the majority of the lakes [4], rivers [5], and coastal ecosystems [6] are severely polluted with metals like Cd, Cr, Cu, Pb, and Zn as well as metalloids like As and Hg. Sources of these metals or metalloids are effluents discharged from large- and small-scale industries, nutrient enrichment, and other domestic and sewage discharge.

Jharkhand is one of the richest areas in the whole country, rich in minerals deposit and forests. The region has huge reserve of coal, iron ore, mica, bauxite and limestones and considerable reserves of copper, chromite, asbestos, kyanite, china clay, manganese, dolomite, uranium etc. [7]. Approximately 52% of India's primary energy needs were met by coal. India is the third-largest producer of coal in the world, behind the US and China. The production of electricity is required to burn the coal. Coal is a chemical source of primary is used in the manufacture of fertilizers, insecticides, medications, and other products [8]. Nonetheless, coal mine workers are at risk for a variety of occupational hazards, including respiratory conditions, musculoskeletal injuries, and time-loss incidents. Jharkhand has nearly 27.3% of India's coal reserves, and a substantial part of the state's economy is dependent on coal mining, which, however, presents various

health and safety hazards to the workers, where lack of effective government funding and safety practices make the situation worse due to industry [9, 10].

It should be noted that most of the previous studies focused on water and sediment analysis but there was a scarcity of analysis of toxicity of metal or metalloids due to consumption of edible fish species in such a non-vegetarian district in Jharkhand. Among the aquatic organisms, fish is a major constituent of a well-balanced diet with a healthy energy source offering low cholesterol levels, high-quality proteins, omega-3 fatty acids, vitamins, and other vital nutrients [11, 12]. India is ranked second in the world's fish production by contributing approximately 6.3% of the fish production. Indian aquaculture is diverse in fish species and pisciculture systems like ponds, lakes, rivers, aquariums, and artificial tanks. According to Raja *et al.*, 2022 [13], aquaculture provides job opportunities to more than 14 million of the population and contributes more than half of the country's annual fish production. Also, fish is the most accepted Indian food and contributes more than 12.5% to the average animal protein source [14]. On the other hand, exposure of metals or metalloids resulted in toxicity and many diseases in fish [15]. Consumption of these metal and metalloid contaminated fish may cause health effects, such as developmental retardation, several types of cancer, kidney damage, endocrine disruption, immunological, neurological effects, and other disorders [16]. However, there is a concern that heavy metals accumulated in edible fish may represent a health risk, especially for populations with high fish consumption rates [17]. The presence of toxic heavy metals in fish can invalidate their beneficial effects. However, fish typically accumulate heavy metals from food, water, and sediments [18, 19]. Therefore, fish are good indicators of the heavy metal contamination levels in aquatic systems [20], because the metal levels in fish usually reflect the levels found in sediment and water of the particular aquatic environment from which they are sourced [21], and time of exposure [22].

Heavy metals can enter into the fish body through feed, water uptake for respiration or ion exchange through semi-permeable membrane followed by accumulating in various tissues within the body [23, 24, and 25]. Fish are considered as good source of protein and fatty acids for human and as heavy metals can be accumulated within the fish body, they can easily be transmitted to the human body and can cause deleterious effects. Like higher vertebrates, fish responds in a similar way to toxicants and can be used to test heavy metal toxicity that are potentially mutagenic, carcinogenic and teratogenic to human beings [26]. These metals can bind with the biological particles containing nitrogen, sulfur, oxygen etc. thus affecting/altering the structure and function of proteins, enzymes, and hormones etc. which ultimately damage different organs of fish [27]. Blood is an important biological component and comparatively easy to detect any changes/alterations in the hematological parameters (RBC, WBC, Hb, Glu etc.), enzyme and hormone levels (ALT, AST, GST, Cortisol etc.) within the fish body due to heavy metals exposure.

Generally, humans are exposed to these metals by ingestion (drinking or eating) or inhalation (breathing) [28]. The harmful effects of environmental pollutants on the human body [29]. Most health risk assessments rely on deterministic methods [30, 31], and the assessment of the impact of pollutants on the human body is relatively simple. In contrast, due to the uncertainties in the selection of parameters and probability models and the hazard identification approach of heavy metal elements [32, 33], uncertainty must be comprehensively considered in the risk assessment.

This case study aims to track health-related issues and estimate the assigned daily task. The Relationship between surface water and coal mining sedimentation. To examine the microstructure for each day that coal mineral resources are impacted.

2. MATERIALS AND METHODS

2.1. Locations of sampling

Coal with deep pockets is well-known in the Damodar River basin. It is abundant in ores and minerals other than coal, including bauxite, limestone, mica, and clay. Based on mining-related enterprises and activities, particularly coal mines, five dams were chosen for this study. While the first two sampling locations, S1 and S2, are located distant from the industrial zone, S3 is located in the center of a large colliery. The average exposure area of industries encompasses the remaining two sites, S4 and S5. These dams are crucial for the native people's navigation, irrigation, hydropower, fishing,

flood control, and water supply. It is discovered that the five sample locations are located in either the Damodar's main stream or one of its tributaries. It is noted that either two thirds of the river basin are located in West Bengal and one third is in Jharkhand. The sampling sites were as follows:

- Konar Dam (S1)
- Maithon Dam (S2)
- Panchet Dam (S3)
- Tenughut Dam (S4)
- Tilaya Dam (S5)

2.2. Estimated Daily Intake calculation

The heavy metal consumption by daily is determined the metal concentration of mean is represent mg kg^{-1} fresh and intake of 15 g of fish by daily. The EDI for heavy metals is influenced by both the quantity of fish eaten and the metal concentrations in fish. Equation (1) was used to get the adult EDI value, per [34].

$$EDI = C_{\text{metal}} \times D_{\text{fish intake}} / B_w \quad (1)$$

where W_{fish} is the daily intake of fish (measured in grams/day), the heavy metal concentration of fish is C_{metal} (measured in $\mu\text{g/g}$), and B_w is adult's body weight (measured in kilogram). The EDI is the daily intake of heavy metals from individual fish (measured in $\mu\text{g/kg/ day}$).

Average of B_w utilized for an Indian male was 52 kg [35]. The value of w_{fish} in the current study was 15g per day. The conduction of survey in the research region to collect this data. Thirty individuals between the ages of thirty and fifty were asked how much fish they ate on a daily basis at each of the six locations. 150 participants were effectively questioned because each participant represents an average household of five. Fish consumption was estimated daily and for each participant on an average basis.

2.3. Pearson's correlation coefficient

One of the techniques used in statistics for a wide range of relationships between Variables is Pearson's correlation coefficient. To assess the degree of correlation between two vectors, one uses the data's covariance matrix. Between two vectors &, the standard Pearson's correlation coefficient is:

$$r = (\sum ((X_i - \bar{X})(Y_i - \bar{Y}))) / (\sqrt{(\sum ((X_i - \bar{X})^2) * \sum ((Y_i - \bar{Y})^2))}) \quad (2)$$

Where: X_i represents the individual values of variable X

\bar{X} represents the mean of variable X

The Pearson's correlation coefficients for the sample as well as population are both less than or equal to 1. When a sample correlation is used, correlations are equal to 1 or -1, which corresponds to data points that are completely supported by a line or to a bivariate distribution with all of its data points accurately on a line (in the case of the population correlation). There is symmetry in the Pearson's correlation coefficient.

2.4 Histopathology of the fish

The following protocol was followed for histopathology. Fixing the gills, liver, kidney, and muscle tissues in serum took four to six hours. Glacial acetic acid, absolute alcohol, and formaldehyde were present in the fixation solution in the following amounts: 60 milliliters, 30 milliliters, and 10 milliliters, respectively. Tissue slices were fixed, tagged, and then gradually dried out with increasing amounts of alcohol. After cleaning and embedding tissue samples in paraffin wax molds, the samples were baked at 58 °C for 45 to 60 minutes. After being taken out of the bubbles, wax blocks with tissues embedded in them were frozen to harden them. With the source of the microtome, the 2.5-micron segment cut into tissues. The tissues are heated in oven at 37 °C for 1 day. After this process, the formation of stained and contrasted using hematoxylin stain and eosin. Before spending a night in the incubator, the cover slip was placed on slides and these portions were mounted with *Canada balsam*. It is examined using the magnification of 10X–60X microscope.

3. RESULTS

3.1. Estimation of Daily Intake (EDI) of heavy metals by Fish consumption

The technique of this research areas indicates that an average of 15 g of fish are consumed daily by each individual. The investigation of this research work is representative the level of the volume of concentration in heavy metal. If the consumes fish sample, the EDI of heavy metal adult who eats fish are consumed. Additionally, PTDI data that were advised by JECFA (1999, 2003) are included in the table. The Cr of 3 $\mu\text{g/kg/day}$ and Sr of 600 $\mu\text{g/kg/day}$ is chosen as the PTDI value in the current investigation due to the absence of available PTDI data for total Cr and Sr. The consumption of fish is EDI heavy metals indicates that consumers are not at risk for health issues from consuming typical amounts of these fish, as the results are below WHO, the dependent of adult person of body mass consumption limits for heavy metals. The JECFA recommended provisional daily intake (PTDI) for As (3.39%), Cd (0.89%), Cu (0.30%), Fe (3.78%), Pb (0.98%), Ni (27%), Zn (1.51%), Cr (7.54%), Co (0.01%), & Sr (2.06%) based on the findings of the current study.

Fish species *P. indicus* (F1), *M. gulio* (F2), *P. conchoni* (F3), *L. calbasu* (F4), *L. rohita* (F5), and *L. bata* (F6) are Pb, Co, Cd, and As found at low dietary intake. The maximum EDI observed only accounts for 0.11 $\mu\text{g/kg}$ for F1 at dam 3, 0.35 $\mu\text{g/kg}$ for F3 at dam 3, 0.22 $\mu\text{g/kg}$ for F3 and F6 at dam 2, and 0.17 $\mu\text{g/kg}$ for F1 at dam 3, respectively. On the other hand, Zn, Sr, Ni, Fe, Cr, and Sr are found to have quite high EDI levels where the corresponding EDI values are 27.39 $\mu\text{g/kg}$ for F1 at dam 4, 31.41 $\mu\text{g/kg}$ for F1 at dam 2, 5.38 $\mu\text{g/kg}$ F1 at dam 3, 56.58 $\mu\text{g/kg}$ F1 at dam 4, 36.31 $\mu\text{g/kg}$ F1 at dam 3, and 2.05 $\mu\text{g/kg}$ F1 at dam 4.

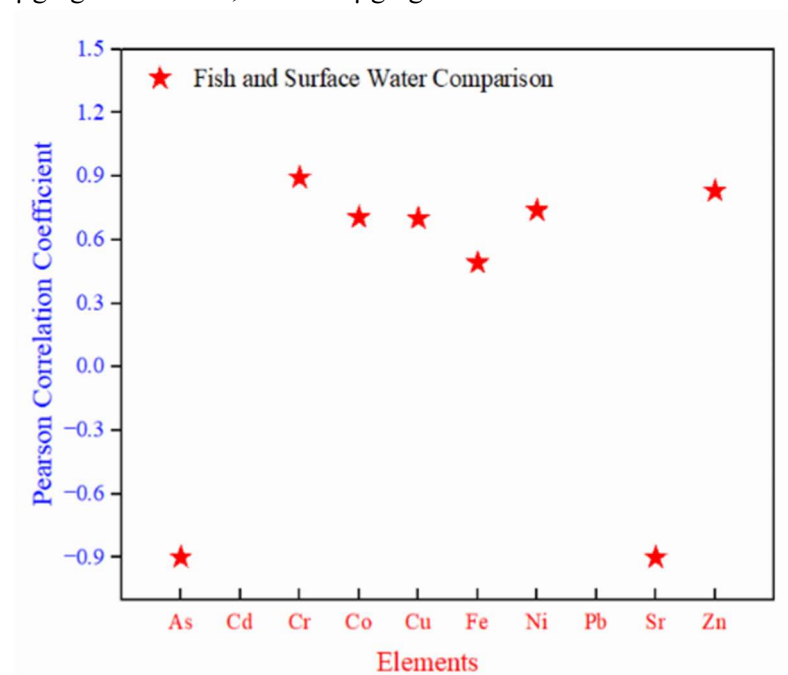


Figure 1. Fish and surface water were compared by using the Pearson correlation coefficient.

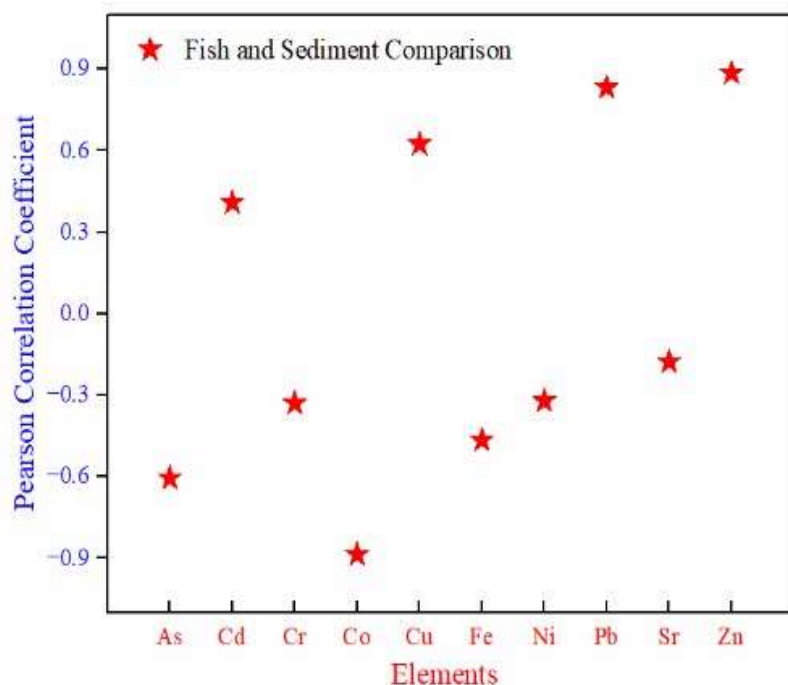


Figure 2. Fish and sediment compared by using person correlation coefficient.

There are recognized links between the heavy metals to evaluate the relationship among the matrices at different, including fish, water, and sediment. The Pearson product moment coefficient values are shown in Figure 1 and 2. The elements Cu, Ni, Cr, Fe, Zn, and Co in water and fish samples, as well as Pb, Cu, and Zn in sediment and fish samples, showed a substantial association, according to the data. Cadmium was not found in water samples, and there was no obvious correlation between fish and sediment samples. This may be explained by the fact that the predominant scavengers of cadmium, a metal that is extremely mobile and possibly bioavailable. It is the minerals of nondetriral carbonate, compounds of organic, and minerals of iron-manganese oxide.

3.2. Histopathology of the fish

Many toxins have raised serious issues for the aquatic ecosystem. Fish have been subjected to numerous biochemical assays in order to assess the consequences of aquatic pollution. However, histopathology provides definite and reliable evidence by highlighting the cellular damage. Therefore, the current study attempts to examine the histopathological alterations in several organs in many fish species under various environmental conditions. It also offers a technique for determining the detrimental effects of pollution in the tissues of different fish species. The most notable histocytopathological alterations in different fish organs that have been used as monitoring instruments in a number of pollution monitoring programs are examined in the current study. We believe that our work may provide a robust and useful method for assessing biological effects and identifying target organ toxicity.

3.2.1 Gills

In toxicant gaseous exchange and osmoregulation, histopathological analysis is a sensitive tool. In addition to providing early warning indications of sickness and homeostasis, the fish gills also play a role in impact assessments that show the effects of toxic elements on fish. As a result, many contaminants get into touch with tissues, cells, or organs, causing damage. Affected gill epithelium undergoes such structural alterations, which is harmful. A toxicant's concentration and length of exposure do, however, affect the degree of damage. Another more typical association of lesions is with metal. In response to a broad spectrum of contaminants, different xenobiotics, and impurities generate different gill lesions. Even though they are concentration dependent, the majority of reported gill modifications in the

literature are actually non-specific and unrelated to the kind of toxin, exposure intensity (acute or chronic), exposure environment (freshwater or ocean), or fish species are shown in Figure 3 to 7.

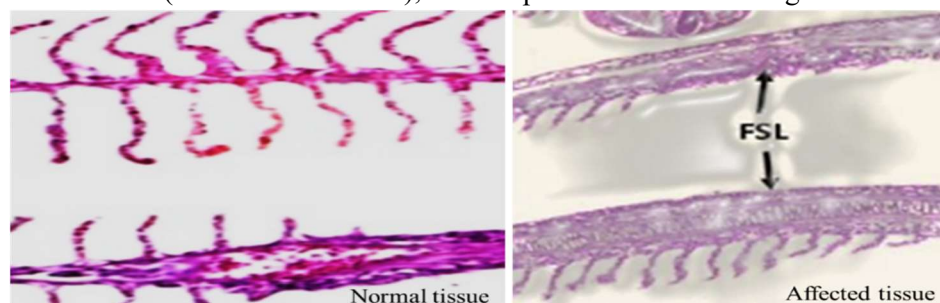


Figure 3. Schematic representation of the Fusion of Secondary lamellae (FSL) of the fish gill.

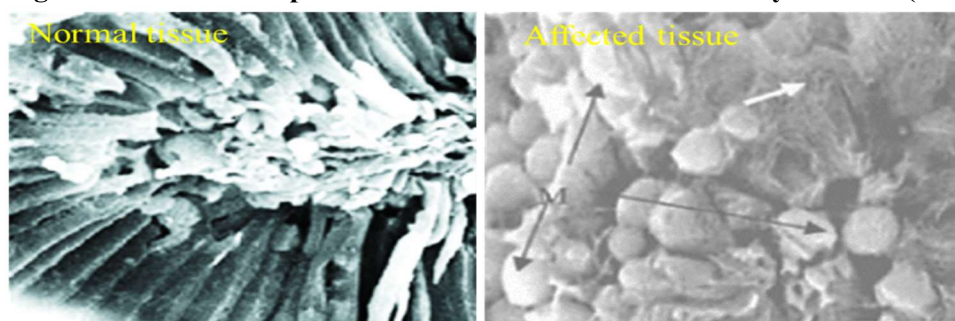


Figure 4. Bulging of the primary epithelium of collected fish gill from the affected zone.

Without a doubt, the host's respiratory ability benefits from the respiratory epithelium alterations. Although moderate alterations don't directly cause fish mortality, they may adversely impact the fish's ability to function. On the other side, serious or substantial damage may result in immediate demise. Overall, the assessment of water quality and the degree of environmental pollution can be done using gills histopathology as a direct and promising indicator.

Gills serve as a barrier to poisons, reducing their absorption and their amplification in other organs. Gills are directly exposed to pollutants as a result of constant contact with water. Fish samples from the Damodar River basin showed signs of gill hypertrophy, vacuolization, and secondary lamellae fusion. Fish taken from the basins of Damodar River showed uplift of primary epithelium and fused gill lamellae as well as inflammatory cell infiltration and hypertrophy.

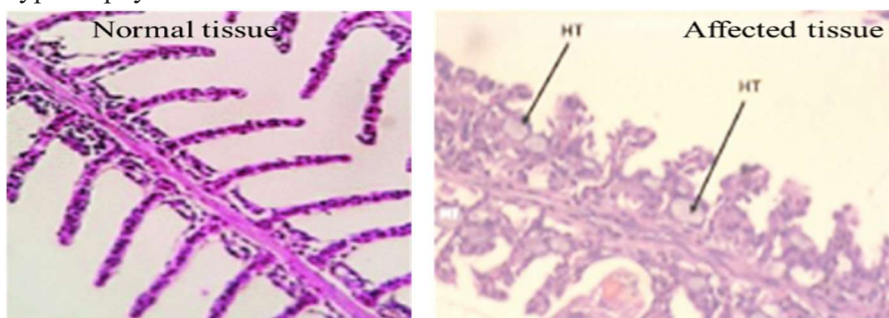


Figure 5. Hypertrophy (HT) zone of fish gill collected from the affected zone.

After being exposed to carbaryl for 96 hours, the fish showed noticeable ultrastructural alterations in their gills, although they still appeared to be in good health. This implied that, despite substantial pathology, there must be a significant amount of redundancy in the transport area within the gill architectural morphology to permit critical respiratory transport. When exposed to carbaryl, fish utilized more oxygen than the control group. Furthermore, after being exposed to carbaryl, the clams' gills were the organs most badly impacted. The histopathologic response primarily comprised of gill epithelial tissue necrosis, which matched the epithelial sloughing we saw by SEM in fish gills. Additionally, Gill

and Asemiates observed that light microscopic evidence of gill epithelial separation and subsequent lamellar fusion was seen in bony fish when carbaryl was induced. A clear increase in the number of mucous cells was seen as a pathologic consequence of the overpopulation stress, as evidenced by the SEM analysis of produced mucus strands on the surface of fish parallels treated with carbaryl.

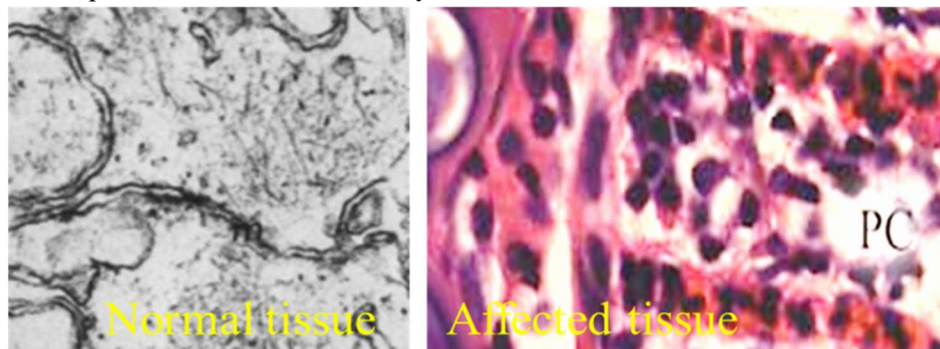


Figure 6. Schematic representation of the freshwater fish of gill tissues with pillar cells (PC).

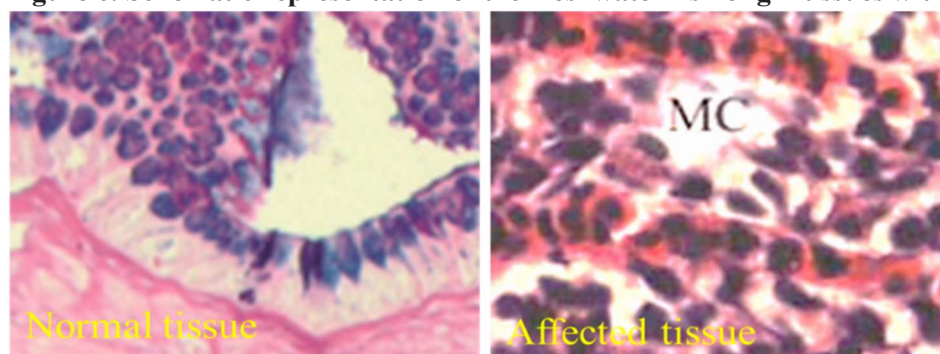


Figure 7. Freshwater fish displaying typical gill tissues with mucus cells (MC).

One of the first injuries to be discovered in fish is the lifting of the respiratory epithelium; this injury is characterized by displacement of the lining epithelium of the secondary lamellae, in which the formation of a space known as edema occurs. This is linked to the presence of chemical contaminants and a decrease in the surface area of the gills. The gill lamellae of the studied individuals also demonstrated a higher percentage of vascular alterations in specimens that were classified as aneurysms. The rupture of pillar cells results in an accumulation of blood in the secondary lamellae, which in turn increases blood flow and causes bleeding, which is the hallmark of an aneurysm.

3.2.2 Liver

One important organ that carries out metabolic processes is the liver. The liver develops various aberrations as a result of the accumulation of heavy metals. Hepatocyte degradation and an increase in mitochondria were shown by the histopathology of the liver from the specimen. The same tissues displayed sinusoids that were dilated and necrosis. Fish specimens that were taken from the impacted area likewise displayed vacuolization in the hepatocytes. Hepatocytes, hepatic arteries, and bile collecting ducts in control fish specimens were all in good condition and had prominent lobules. 80% of seafood meals are made up of muscles. Because they are not directly exposed to contaminants, muscles were shown to be less impacted.

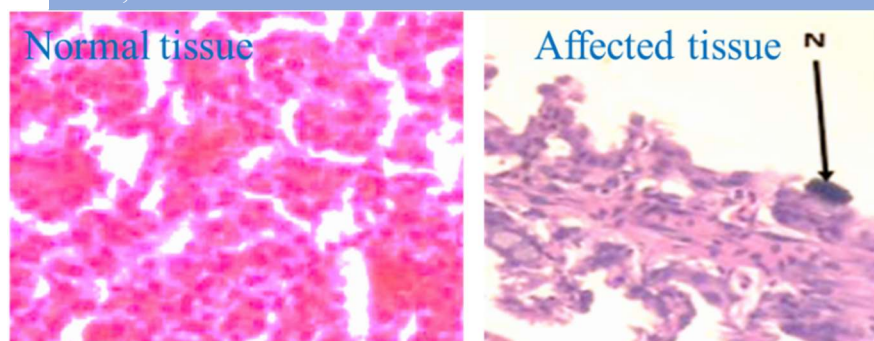


Figure 8. Graphical representation of the fish collected from the affected area in liver tissues with necrosis (N).

Fish muscle tissues that were transversely sectioned and collected revealed oedema, atrophy, and necrosis. Lesion formation and other histopathological changes are frequent in the liver as a result of toxicant exposure and accumulation. Additionally, contaminants have an impact on the organ's strong metabolic ability. Many researchers employed liver histopathology as accurate indicators of different pollutants. The third category of liver changes, known as inflammatory changes, is thought to be the least relevant predictor of pollutant exposure, albeit this group can reveal more about the general state of the fish's health and welfare.

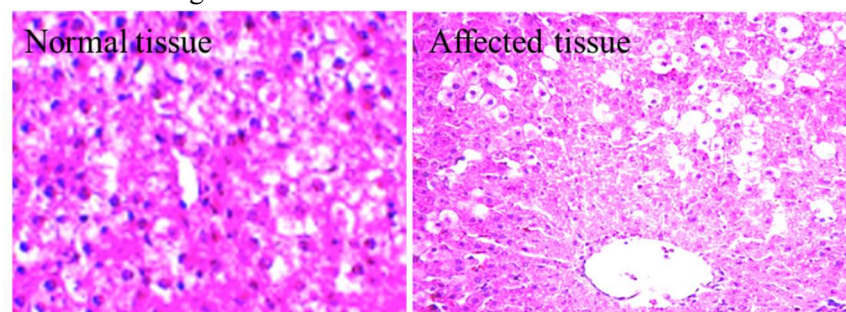


Figure 9. Hepatocytes vacuolization of the fish sample collected from the affected area in liver tissues.

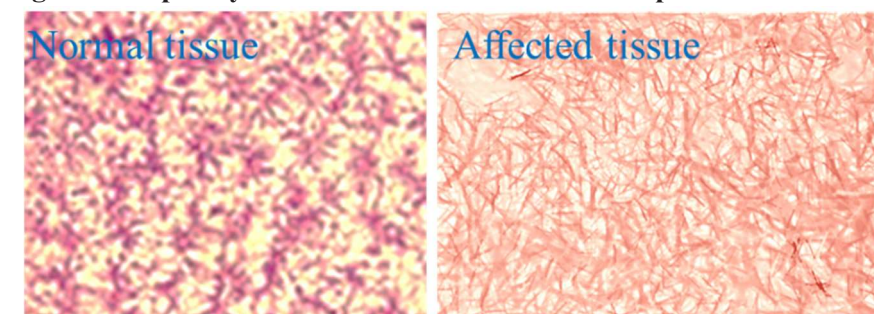


Figure 10. Hepatic artery of the freshwater fish with normal gill tissues.

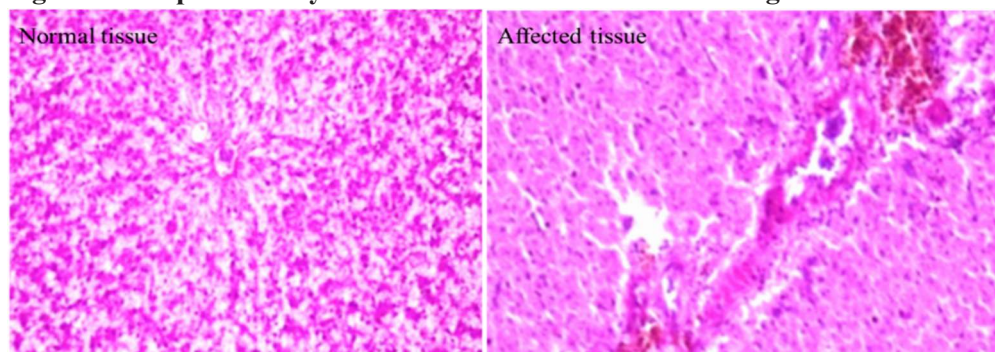


Figure 11. Freshwater fish showing liver tissue cells affected area.

Fish treated to thiobencarb for nine days had severe congestion and hemorrhages in all internal organs, particularly the liver and ovaries, according to histology exams. Histological studies of liver tissues from control necrosis (Figure 8) revealed a typical parenchymatous appearance. Hepatocytes, polygonal cells with a highly pigmented nucleolus and a central, spherical nucleus, comprise the liver. Fish treated to thiobencarb for three days displayed Hepatocyte vacuolization in hepatocytes, and a small infiltration of mononuclear cells in the portal region (Figure 9). Following therapy for nine days, the liver showed fragmented hepatocytes and a small invasion of the Hepatic artery in the portal region (Figure 10). Following a 15-day course of therapy, hepatocytes showed signs of karyopyknotic, including an increase in Melano macrophages and mononuclear cell infiltration in the liver's portal regions (Figure 11).

3.2.3 Kidney

In excretion and homeostasis, the kidneys play a significant role. However, the glomerulus and bowmen space of catfish whose kidney tissues were recovered downstream of CMD were constrained. Upstream specimens' kidney tissues displayed haemorrhaging, damaged renal tubules, and nuclear tubular cells are depicted in Figure 12 to 15. The control specimen displayed normal Bowmen's capillaries surrounding the glomerulus and generating normal glomerulus tufts, as well as normal nephrons with normal renal corpuscles.

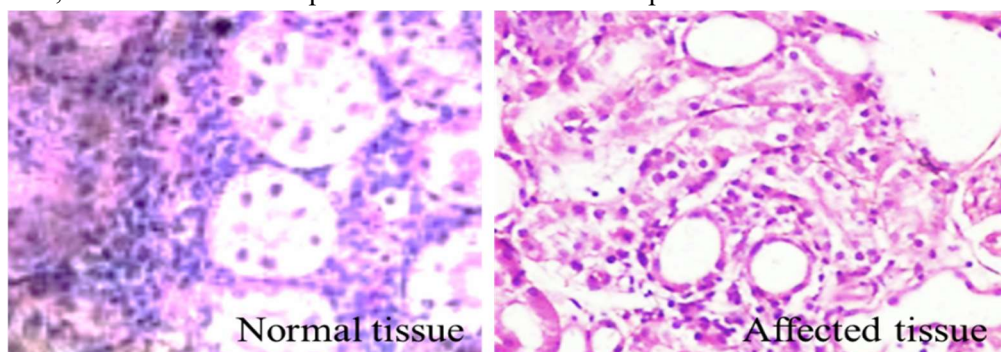


Figure 12. Graphical representation of the kidney tissues with constricted glomerulus.

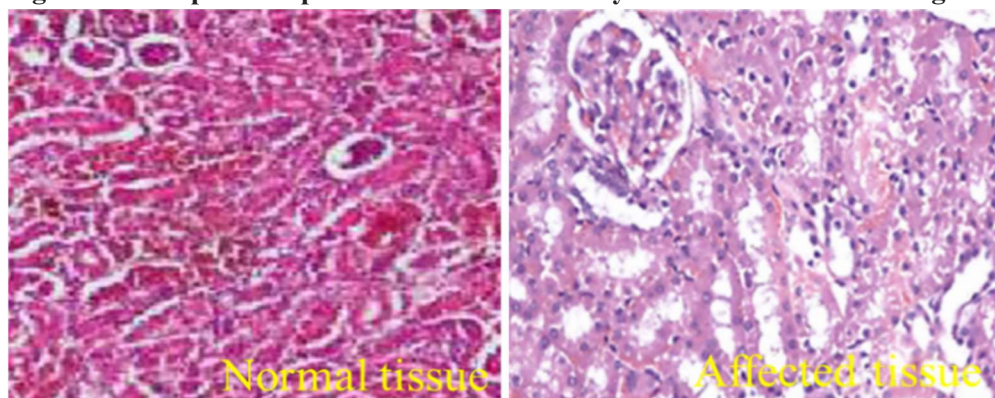


Figure 13. Schematic representation of the kidney tissues with destructive renal tubule.

With the exception of tubule and hematopoietic tissue apoptosis, trunk kidney changes were extremely varied and nearly completely restricted to animals exposed to 5 and 10 lg Cd L⁻¹; however, no clear dose- or time-dependent pattern was seen. Both the control and 0.5 lg Cd L⁻¹ fish had kidneys that were structurally normal throughout, with discrete lumens formed by ciliated cuboidal epithelial cells (eosinophilic cytoplasm) lining well-defined tubules. Malpighian corpuscles were tiny and infrequent, as would be expected in a marine teleost, making them insufficient for a thorough histological examination of the glomeruli. Animals exposed to the highest amounts frequently experienced hyperaemia, which suggests some degree of inflammation. There were rare instances of localized hemorrhage due to the bursting of blood-congested arteries (in fish exposed to 10 lg Cd L⁻¹ alone). Melano macrophages often invaded adjacent vascular segments in the direction of apoptotic foci, sometimes creating dense centers. The majority of kidney tubule

abnormalities were epithelial cell vacuolation and necrosis. Regarding the liver, unambiguous nuclear pleomorphisms were uncommon and did not explain any discernible pattern.

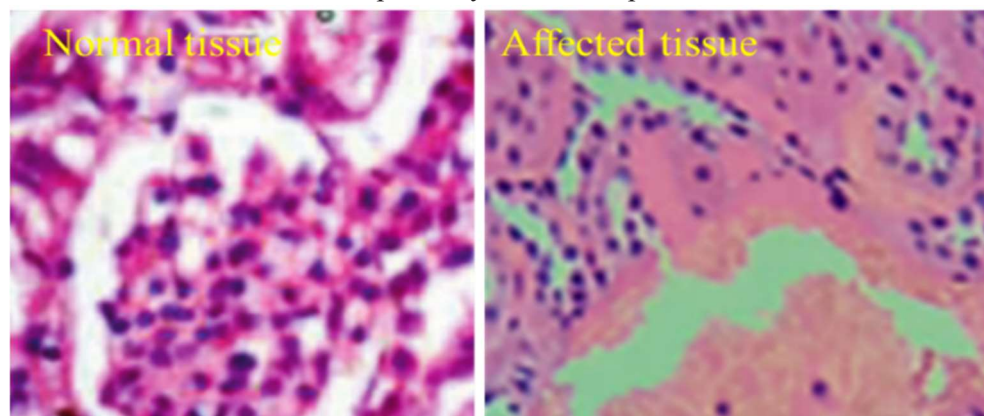


Figure 14. Fish from affected area showing damage in kidney tissues with hemorrhage.

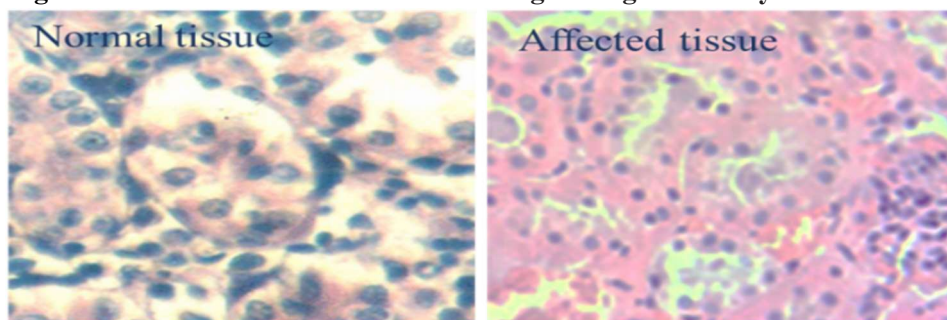


Figure 15. Proximal convoluted tubules of the kidney tissues in the fish.

3.3 SEM analysis of the fish from affected area

Gold-mercury amalgam grains can be seen in photomicrographs taken with a scanning electron microscope in secondary electron mode. Figures 16 (a-d) dredge test samples from the Dhanbad mining sites taken in 2022. Scale bars are in micrometres (m). A composite of amalgam granules measuring 700 by 1,000 m (a), taken from the third session of the dredge test; (b) a close-up of a smooth amalgam surface, with a texture that may be a pseudomorph of gold crystals measuring 100 m in diameter, taken from the first two hours of the dredge test; (c) a botryoidal texture on the amalgam surface, taken from the first two hours of the dredge test; and (d). Sample from Figure 16 (e-h), bedrock in contact with spherical liquid elemental mercury (Hg (0)) beads. (e) Composite of amalgam grains, roughly 700 by 1,000 m; (f) Hg (0) beads, roughly 0.5 and 2 m in diameter; (g) Hg (0) beads, roughly 5 m in diameter; and (h) Hg (0) beads, roughly 2 m in diameter.

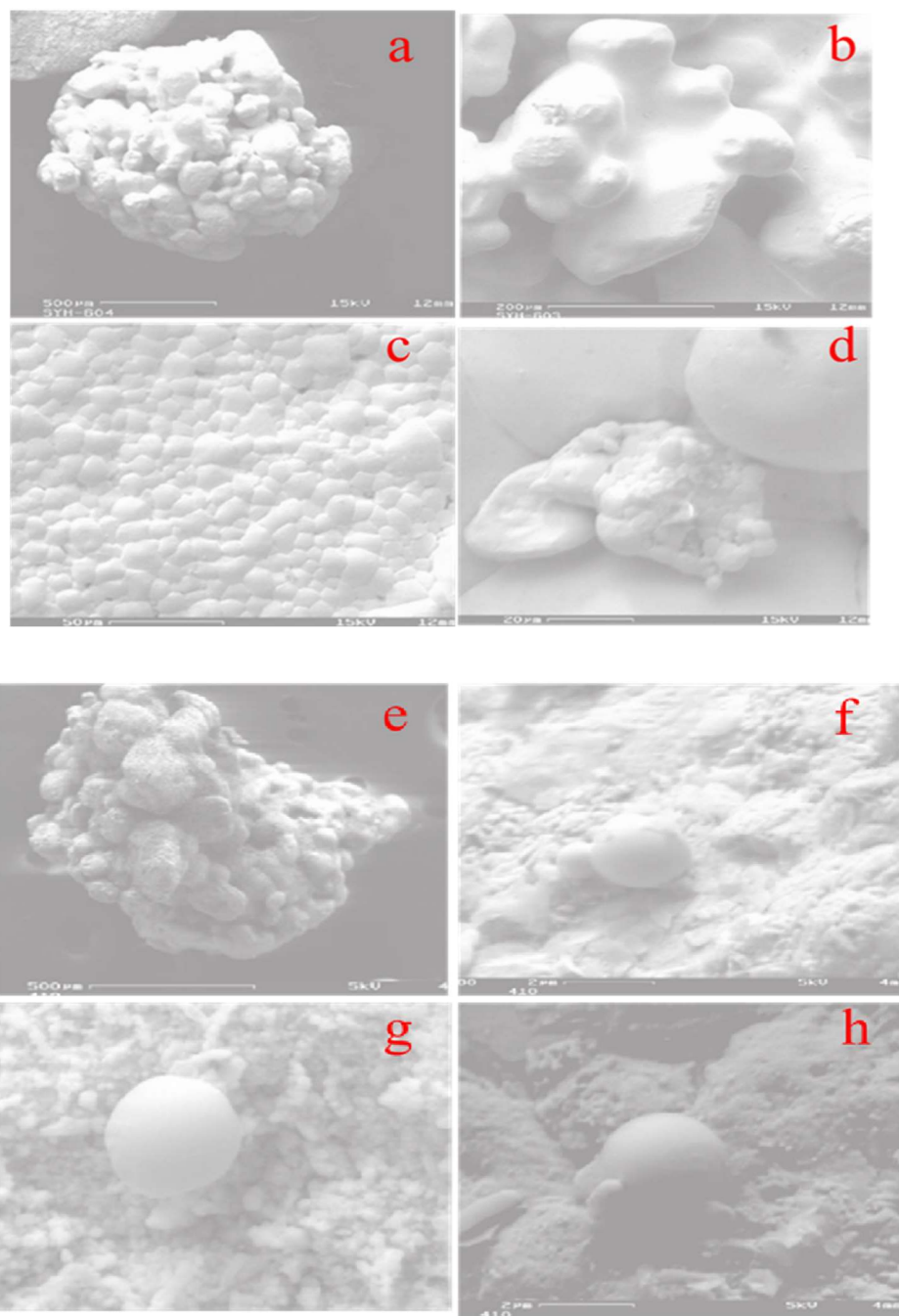


Figure 16. SEM images of fish collected from affected zone.

4. Discussion

Tong et al., 2020 [36] investigated the risk of human health issues from the process of power generation from coal-fired station. The risk of health issues was highest for workers in the ash removal industry ($4.08 \times 10^{-6} \pm 2.85 \times 10^{-6}$ (95% CI)), then for workers in other employment groups. **Prasad et al., 2021 [37]** studied on the prevalence of lung function decline in coal miners and whether lung function markers and dust exposure in 230 workers from an underground tunnel in West Bengal, India, and 130 age-matched quasiworkers. There was a significant (p 0.050) difference in lung function indices between the non-exposed (23.85%) and exposed (43.91%) groups. **Zhang et al., 2016 [38]** evaluated the assessment of the risk that mining operations provide to human health through the drinking water

channel was done using the United States Environmental Protection Agency's (USEPA) assessment approach. The investigation showed that the carcinogenic risk values were all higher above the highest allowable threshold set by the USEPA, ranging from 1.05,105 to 3.5, 104. Chromium's carcinogenic potential accounted for 99.67% of the total carcinogenic risk. **Masto et al., 2021 [39]** studied to determine the composition and possible health risks, Potentially Toxic Elements of (Pb, Ba, Zn, Cu, Sr, Cr, Ni, As, and Co) are found in dust road in the area of coal mining (Dhanbad, India). The metals are Zn (224), Pb (128), Cr (45.2), Ni (22.0), As (17.5), Cu (52.6), and Co. (8.11) showed that the maximum concentration of PTE mean, with Ba having the highest. The range of index in overall load pollution is 0.43 to 1.0. At the study site, PTEs are Zn, Fe, Mn, Co, and Pb obtained in the road dust. **Lakra et al., 2019 [40]** studied on the concentration of heavy metals Fe, Zn, Cu, Mn, Ni, Cd, Pb and Cr were analyzed in the water and various tissues of edible catfish *Clarias batrachus* reared in a pond receiving effluents from Rajrappa coal mine, Jharkhand, India. Results showed that accumulation of metals in fish tissues were in the following order: liver > kidney > air breathing organ (ABO) > gills > skin > brain > muscles. Among the various tissues the highest accumulation of most of the metals was recorded in the liver (2.05–271.28 mg/kg dry weight) and lowest in the muscles (1.39–30.27 mg/kg dry weight), while the concentration of metals in other tissues ranged in between.

Lakra et al., 2021 [41] investigated the toxicity of the coal mine effluent (CME) generated at the Rajrappa coal mine on the catfish *Clarias batrachus*. The results of metal bioaccumulation in CME-exposed fish tissues revealed the highest metal concentration in liver (1.34–297.68 mg/kg) while lowest in muscles (1.47–23.26 mg/kg) as compared to other tissues and so was the metallothionein level. **Bharti and Banerjee, 2011 [42]** studied on Metal accumulation in various tissues of *Heteropneustes fossilis* exposed to the effluent generated from an open cast coal mine. Out of the eight metals investigated, accumulation (mg kg⁻¹ dry weight of tissue) of Fe was maximum in every tissue followed by liver (265.88 ± 49.89) [kidney (153.0 ± 65.85) [gills (50.66 ± 23.923) [brain (49.303 ± 5.11) [air breathing organs (27.98 ± 10.93) [skin (19.56 ± 2.53) [muscles (8.74 ± 0.83). This was succeeded by Pb in brain (39.35 ± 5.79), Zn in kidneys (27.04 ± 2.31), Mn in the gills (20.69 ± 3.044), Cu (12.53 ± 1.01) [Cr (5.10 ± 2.87) in liver and Cd in kidneys (2.18 ± 0.084).

5. CONCLUSIONS

Fish gills are essential to their respiratory, osmoregulatory, and excretory systems. Because of the high rate of absorption through gills, fish are a sensitive target for its toxicity. According to this study, the gills experienced desquamation, necrosis, elevation of the lamellar epithelium, oedema, hyperplasia of the epithelial cells, aneurism, and fusion of the secondary lamellae following exposure to heavy metals.

One of the most significant advantages of using histopathological sensors in environmental screening is the capacity to look at certain target organs, such as the liver, kidney, and gills. Still, the fish are feeling the direct effects of stress in addition to the indirect effects of the contaminants. Based on available data, histopathological alterations may serve as useful indicators for field assessment. The fish that reside in the impacted area have a tiny quantity of mercury deposition in their bodies, according to the SEM examination of the fish. Long-term exposure, even if it stays within the allowable limit, can result in significant morphological changes and the extinction of fish species, which are the main food supplies for the local population.

DECLARATIONS:

ETHICS APPROVAL

No ethics approval is required.

HUMAN AND ANIMAL ETHICS:

Not Applicable.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

AUTHOR'S CONTRIBUTIONS

Author 1: Moumita Ray Mukherjee

She performed the conceptualization, Methodology, Data collection and writing the study

Author 2: Dr. Divya Shrivastava

She analysis the dataset and conceptualization in the study.

Author 3: Dr. V. A Selvi

She Performed the Analysis of overall concept, writing and editing.

Author 4: Dr. Rama Sadashiv Lokhande

She analysis the paper and supervisor of this paper.

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The authors declare that we have no conflict of interest.

COMPETING INTERESTS

The authors declare that we have no competing interest.

INFORMED CONSENT

Not Applicable.

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Not Applicable

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